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ANALYSIS OF HEARING THRESHOLDS IN PATIENTS WITH HEARING IMPAIRMENT ASSOCIATED WITH THE *GJB2* (Cx26) GENE MUTATIONS IN BURYATIA

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In this work, the analysis of the hearing condition in 26 patients with hearing impairment, in whom biallelic mutations of the GJB2 gene (Sh26) were detected in the Republic of Buryatia, was carried out. Genotype-phenotypic comparisons showed that for all 7 GJB2 genotypes: c.[35delG];[35delG]; c.[-23+1G>A];[35delG]; c.[-23+1G>A];[-23+1G>A]; c.[-23+1G>A];[516G>C]; c.[-23+1G>A];[327_328delGGinsA]; c.[35delC];[299_300delAT]; c.[235delC];[235delC] congenital (detected before one year – 76.0%), symmetrical (84.6%), sensorineural (100.0%) hearing loss is detected, variable in severity both between different GJB2 genotypes and within the same GJB2 genotype (grade III in 9.6%, grade IV in 17.3%, deafness in 73.1%). Based on the median hearing thresholds PTA0.5,1.0,2.0,4.0 kHz, three phenotypes were identified - "mild phenotype", "medium phenotype" and "severe phenotype". We conducted a genotype-phenotypic comparison, as a result of which GJB2-genotypes c.[-23+1G>A];[-23+1G>A] and c.[-23+1G>A];[516G>C] were assigned to genotypes with “average” (median 66.875 dB and 64.375 dB, respectively), and the remaining GJB2-genotypes: c.[35delG];[35delG]; c.[-23+1G>A];[35delG]; c.[-23+1G>A];[327_328delGGinsA]; c.[35delC];[299_300delAT]; c.[235delC];[235delC] - to genotypes with a "severe" form of the phenotypic effect. No genotypes with a "mild" phenotypic effect have been identified. At the same time, the hearing thresholds for GJB2 genotypes with the "average" form of the phenotypic effect (c.[-23+1G>A(;)-23+1G>A] and c.[-23+1G>A(;)516G>C]) were significantly (p=0.02268) better than in the reference group with GJB2 genotype c.[35delG];[35delG].

Keywords: deafness, GJB2 gene, genotype-phenotypic analysis, degree of hearing loss, Republic of Buryatia.

Introduction. It is known that up to 50% of cases of congenital deafness have a hereditary etiology [10]. At the same time, the genetic causes are extremely diverse, but the proportion of mutations in the *GJB2* gene (13q11-q12), encoding the protein of intercellular gap junctions - connexin 26 (26 kDa) [8], is significant and averages about 17.3% [5]. With the understanding of the etiology and pathogenesis of autosomal recessive deafness type 1A, studies aimed at genotype-phenotypic comparisons of hearing impairment in patients depending on the nature of mutational damage in the *GJB2* gene have become relevant. At the same time, of all known mutations of the *GJB2* gene, genotype-phenotypic studies mainly concerned the most common c.35delG mutation in Europe in the homozygous and compound-heterozygous state with other rarer pathogenic variants of the *GJB2* gene, as well as c.235delC and p.Val37Ile mutations common in Asia [3, 6, 15]. These studies have identified *GJB2* genotypes with a relatively "mild" phenotype. Thus, hearing loss in patients with genotypes: p.[Val37Ile];[Val37Ile] (median PTA 27dB), p.[Met34Thr];[Met34Thr] (median PTA 30dB) and p[Met34Thr];[Val37Ile] (median PTA 23dB) was significantly less than in patients with more “severe” genotypes – c.[35delG];[35delG] (median PTA 102dB), c.[35delG];[del(GJB6-D13S1830)] (median PTA 108dB) (p<.0001) and c.[235delC];[235delC] (median PTA 100.68dB) [6, 15]. In general, the authors concluded that the so-called truncating mutations (deletions, nonsense mutations) lead to greater hearing loss than non-truncating mutations (missense mutations) [9]. Genotype-phenotypic comparisons were also made for the homozygous *GJB2* genotype c.[-23+1G>A];[-23+1G>A], among deaf patients in Yakutia [1].

In 2021, studies were conducted aimed at diagnose of hereditary non-syndromic hearing loss in the Republic of Buryatia (Eastern Siberia). Using direct sequencing, in 165 individuals with hearing impairment the spectrum and frequency of mutations in the *GJB2* gene were determined. In the studied sample, 13 known allelic variants of the *GJB2* gene were found (c.-254C>T, c.-49G>A, c.-23+1G>A, c.35delG, c.79G>A, c.101T>C, c.109G>A, c.235delC, c.299_300delAT, c.327_328delinsA, c.341A>G, c.457G>A and c.516G>C). In general, the contribution of biallelic mutations of the *GJB2* gene to the etiology of hearing loss in the total sample of patients in Buryatia was 15.8% (26/165) [2]. Following the general trends in genotype-phenotypic comparisons, the purpose of this study was to analyze hearing thresholds in individuals with biallelic mutations of the *GJB2* gene in Buryatia.

Material and methods. *Study participants.* Informed voluntary examination by an audiologist-otorhinolaryngologist was performed by 165 deaf people. An examination of the ENT organs, audiometry, blood sampling from the cubital vein for molecular genetic analysis were carried out.

Audiometry. Pure tone audiometry (PTA) was performed using a AA222 tympanometer-audiometer (Interacoustics, Denmark) for air conduction at frequencies of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and for bone conduction at frequencies of 0.25, 0.5, 1.0, 4.0 kHz in steps 5.0 dB

DNA diagnostics. Samples (n=165) of genomic DNA were extracted from leukocytes. Amplification of the *GJB2* gene fragments, including exon 1 and exon 2 of the *GJB2* gene with flanking regions, was performed by PCR on MJ Mini thermal cycler (Bio-Rad) using the primer sequence described earlier [7, 11, 13]. The determination of the primary nucleotide sequence was carried out on an ABI Prism 3130XL Genetic Analyzer capillary sequencer (Applied Biosystems, USA) (Central Collective Use Center Genomics, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk). The sequence variants were determined by comparison with the reference sequences of the *GJB2* M86849

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gene.2 and U43932.1 (GenBank) using the Chromas software.

Sample. Of the 165 examined deaf patients with hearing impairments, 26 were found to have biallelic mutations in the *GJB2* gene in the homozygous or compound heterozygous state. Among them, 7 different pathological *GJB2* genotypes were identified: c.[35delG];[35delG] (total 16 individuals, of which 9 men, 7 women aged 32 to 76 years, mean age 55± 12.25 years, all Russians), c.[-23+1G>A];[35delG] (total 5 individuals, of which 2 men, 3 women aged 19 to 59 years, average age 47± 17.33 years, all Russians), c.[-23+1G>A];[-23+1G>A] (1 Buryat, 58 years old), c.[-23+1G>A];[516G>C] (1 Buryat, 46 years old), c.[-23+1G>A];[327_328delGGinsA] (1 Buryat, 47 years old), c.[35delC];[299_300delAT] (1 Russian, 65 years old), c.[235delC];[235delC] (1 Buryat, 39 years old). Ethnicity was ascertained using a questionnaire (up to and including the third generation). To compare hearing thresholds, the reference group identified a sample of individuals with the *GJB2* genotype c.[35delG];[35delG] as the most numerous (n=16) and well studied.

Audiological analysis. Audiograms with breaks were normalized by adding maximum values(120.0 dB) in the places where the patient did not respond, according to the recommendations of the International Bureau of Audio Phonologists (www.biap.org.). The type of hearing loss was considered conductive - with an increase in air conduction thresholds on audiograms, sensorineural - with an increase in bone and air conduction thresholds on audiograms, mixed - with an increase in bone and air conduction thresholds with an interval exceeding a total of 20.0 dB in $PTA_{0.5,1.0,\ 2.0,4.0}$ kHz. Hearing loss was considered asymmetric when the difference between the hearing thresholds in the PTA of 0.5,1.0,2.0,4.0 kHz was more than 15.0 dB. The degree of hearing loss was assessed by the average hearing threshold in $PTA_{0.5.1.0.2.0.4.0}$ kHz according to the WHO classification: I degree - 26.0-40.0 dB, II degree - 41.0-55.0 dB, III degree - 56.0-70.0 dB, IV degree - 71.0-90.0 dB, deafness -> 91.0 dB. Phenotypes with I-II degree of hearing loss we attributed to "mild", with III-IV degree of hearing loss - to "medium", and deafness - to the "severe" form of the phenotype. For a detailed audiological analysis, the number of ears was used (26 patients = 52 ears), because the functional abilities of paired organs in humans can be different and the pathological effect of the genotype can be expressed in different ways.

Statistical analysis. StatPlus software (2021 AnalystSoft Inc.) was used for statistical processing. The median hearing thresholds of six *GJB2*-genotypes were compared in pairs with the reference group of the c.[35delG];[35delG] genotype using the Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

Ethical control. The surveys included in the scope of the research work were conducted with the informed voluntary consent of the participants or their parents. This research work was approved by the local committee on biomedical ethics at the "YSC CMP" (Yakutsk, Protocol No. 16 dated April 16, 2009).

Results and discussion. In this study, out of 165 participants with hearing impairment, we selected the audiological data of 26 individuals with biallelic mutations of the *GJB2* gene (Cx26), identified in the homozygous or compound heterozygous state. Among the identified 26 patients, seven different *GJB2* genotypes were found: c.[35delG];[35delG], c.[-23+1G>A];[35delG], c.[235delC];[235delC], c.[- 23+1G>A];[-23+1G>A], c.[-23+1G>A];[516G>C], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT]. Assuming that severe hearing loss after birth would be recognized sooner than those with less severe hearing loss, we conducted a survey about the age at which hearing loss was diagnosed. This criterion can help reveal phenotype variability in different *GJB2* genotypes and even within the same *GJB2* genotype. As a result, it was found that the vast majority of *GJB2*-positive patients had predominantly early detection of hearing loss (up to one year

Table 1

Age of onset of hearing impairment in individuals with biallelic mutations of the *GJB2* gene

GJB2-genotype	Number of patients	Age of onset	Number of patients	Early diagnosis (before 12 mounts)	Late diagnosis. (before 12 mounts)
c.[35delG];[35delG]	16	In 1 years	11	11 68.7% (44.0-85.8%)	5 31.3% (14.2-56.0%)
		In 2 years	1		
		In 3 years	3		
		In 6 years	1		
c.[-23+1G>A];[35delG]	5	Before 12 mounts	5	**5** **100% (54.1-99.6%)**	**0** **0% (0.43-45.9%)**
c.[-23+1G>A];[-23+1G>A]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[-23+1G>A];[516G>C]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[-23+1G>A];[327_328delGGinsA]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[35delC];[299_300delAT]	1	Before 12 mounts	1	1 100% (58.0-98.7%)	0 0% (0.13-84.2%)
c.[235delC];[235delC]	1	In 6 years	1	0 0% (0.13-84.2%)	1 100% (58.0-98.7%)
In total seven *GJB2*-genotypes	In total 26 patients (CI)			**20** **76% (57.7- 88.9%)**	**6** **23% (11.0-42.3%)**

Note: n - number of patients, CI - confidence intervals ($p<0.05$). Statistically significant differences are highlighted in bold.

Table 2

Severity of hearing loss in patients with different *GJB2*-genotypes

GJB2-genotype	Degree of hearing loss	Median hearing thresholds in $PTA_{0.5.1.0.2.0.4.0\ кГц}$	Mann-Whitney U test *p*	Phenotypes
c.[35delG];[35delG] (n=16. 32 уха)	IV (n=5)	115.0	Reference group	Sever
	Deafness (n=27)			
c.[-23+1G>A];[35delG] (n=5. 10 ушей)	IV (n=3)	106.25	0.23097	Sever
	Deafness (n=7)			
c.[-23+1G>A];[327_328delGGinsA] (n=1. 2 уха)	IV (n=1)	96.875	0.23320	Sever
	Deafness			
c.[35delC];[299_300delAT] (n=1. 2 уха)	III (n=1)	92.5	0.24774	Sever
	Deafness			
c.[235delC];[235delC] (n=1. 2 уха)	Deafness (n=2)	117.5	0.24372	Sever
c.[-23+1G>A];[-23+1G>A] (n=1. 2 уха)	**III (n=2)**	**66.875**	**0.02268**	**Median**
c.[-23+1G>A];[516G>C] (n=1. 2 уха)	**III (n=2)**	**64.375**	**0.02268**	**Median**

Примечание. Жирным шрифтом выделены статистически достоверные различия (p<0.05).

of age, inclusive) - 76.0% (CI 57.7%-88.9%), late detection (after one year) was reported in 23.0% (CI 11.0%-42.3%) (p<0.05) (Table 1). With genotypes c.[-23+1G>A];[35delG], c.[-23+1G>A];[-23+1G>A], c.[-23+1G>A];[516G>C], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT] (n=5) hearing loss was detected up to one year (Table 1). Late detection of hearing impairment (at six years) was registered in one case with genotype c.[235delC];[235delC]. The most numerous genotype c.[35delG];[35delG] (n=16) was found to be the most variable in terms of detection of hearing impairment. So, in 11 patients with this genotype, hearing loss was detected before one year, in 5 patients - at two and three years, and in one case at six years. It is interesting to note that in the c.[-23+1G>A];[35delG] genotype, hearing loss was recorded earlier (up to one year - 100.0%, CI 54.1-99.6%) significantly more often than late detection (0%, CI 0.43% - 45.9%) (Table 1). Based on the fact that all patients with biallelic *GJB2* mutations were born before the introduction of audiological screening of newborns and children of the first year of life, it can be assumed that most of the observed *GJB2*-genotypes lead to predominantly severe and, accordingly, early detected hearing loss.

Audiometric data in individuals with pathological GJB2-genotype. The results of tone threshold audiometry showed that all detected *GJB2*-genotypes (100%) were associated with sensorineural type of hearing loss (n=26 individuals, 52 ears). At the same time, the majority in the sample (n=22; 84.6%) had symmetrical hearing loss. The exception was four individuals with different *GJB2* genotypes (15.4%) who, in the absence of a visible otological problem, had asymmetric hearing thresholds. In these patients, the interaural hearing threshold difference ranged from 36.6 dB for the c.[35delG];[35delG] genotype, 20.0 dB for the c.[-23+1G>A];[35delG] genotype, 31.5 dB for the c.[-23+1G>A];[327_328delGGinsA] genotype; up to 45.0 dB for the c.[35delC];[299_300delAT] genotype. In general, in the sample of *GJB2*-positive patients, hearing loss ranged from grade III to deafness: grade III sensorineural hearing loss in 9.6% (n=5 ears), grade IV sensorineural hearing loss in 17.3% (n=9 ears), sensorineural deafness in 73.1% (n=38 ears).

For genotype-phenotypic comparison between identified *GJB2*-genotypes, the c.[35delG];[35delG] genotype was chosen as the reference genotype, as the most well-studied in the world and the most frequent in our sample. In the reference group of patients, the median hearing threshold in the PTA of $_{0.5,1.0,2.0,4.0}$ kHz was at the level of 115.0 dB. The comparison of the median hearing thresholds in $PTA_{0.5,1.0,2.0.4.0}$ kHz of the reference group with other identified *GJB2* genotypes demonstrated comparable hearing thresholds for *GJB2* genotypes: c.[-23+1G>A];[35delG] (median 106 .25 dB), c.[-23+1G>A];[327_328delGGinsA] (median 96.875 dB), c.[35delC];[299_300delAT] (median 92.5 dB) and c.[235delC];[235delC] (median 117.5 dB) (p>0.05). The median hearing thresholds of the reference and these four *GJB2* genotypes corresponded to the degree of hearing loss - deafness. In this regard, these genotypes c.[-23+1G>A];[35delG], c.[-23+1G>A];[327_328delGGinsA], c.[35delC];[299_300delAT], c.[235delC];[235delC], including the reference c.[35delG];[35delG], were classified as genotypes with a severe phenotypic effect (Table 2).

Significantly better hearing thresholds compared to the reference genotype were demonstrated by two *GJB2* genotypes: c.[-23+1G>A];[-23+1G>A] (median 66.875 dB) and c.[-23+1G>A];[516G>C] (median 64.375 dB) (p=0.02268) (Table 2). Median hearing thresholds for these two *GJB2* genotypes corresponded to grade III hearing loss (Table 2). As a result, we assigned these two *GJB2* genotypes c.[-23+1G>A];[-23+1G>A] and c.[-23+1G>A];[516G>C] to genotypes with " average" form of the phenotypic effect. In accordance with the data obtained, no genotypes with a "mild" phenotypic effect, corresponding to I-II degrees of hearing loss, were identified (Table 2).

Previously, it was shown that for patients with the c.[35delG];[35delG] genotype, the median in $PTA_{0.5,1.0,2.0}$kHz was 102 dB [9]. It is likely that the observed difference (~13 dB) in the depth of hearing loss between our study and that of Snoeckx et al. [9] is due to the difference in frequencies taken into account. In Snoeckx et al. study [9], the frequency range was limited to three measured frequencies ($PTA_{0.5,1.0,2.0}$kHz), while in our study, measurements were made at four frequencies ($PTA_{0.5,1.0,2.0,4.0}$kHz). In addition, in this multicenter study, it was shown that with the c.-23+1G>A mutation in the compound with the c.35delG mutation [9] a significantly milder phenotype is recorded. In the present work, it was shown that the c.-23+1G>A mutation in the homozygous state (median 66.875 dB) and in the compound-heterozygous state with the c.516G>C mutation (me-

dian 64.375 dB) also has a significantly (p=0.02268) less hearing loss (moderate phenotype) compared to the phenotypically more "severe" reference genotype c.[35delG];[35delG] (median 115.0 dB) (Table 2). However, it should be noted that earlier on a larger sample of patients with genotype c.[-23+1G>A];[-23+1G>A], with a median of 85.41 dB (which corresponds to IV degree of hearing loss), it was shown wide individual variability of hearing thresholds, ranging from mild hearing loss to deafness [1].

Conclusion. Thus, the analysis of the state of hearing in individuals with mutations in the *GJB2* (Cx26) gene in Buryatia revealed the following:

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ASSOCIATION OF POLYMORPHISMS OF THE GENES *HTR2A* AND *5-HTT* WITH SMOKING IN YAKUTS

Tobacco smoking is the most common form of addiction worldwide. The aim of our study was to determine whether the rs6311 polymorphisms of the *HTR2A* gene and the 5HTTLPR and rs25531 polymorphisms of the *5-HTT* gene are associated with smoking in the Yakut population. The study involved 223 people, including 115 smokers and 108 non-smokers. The results of the analysis of the relationship between the rs6311 polymorphism of the *HTR2A* gene and smoking showed that in the group of smokers, allele A was somewhat more common than in the group of non-smokers (OR - 1.138, 95% CI = [0.742-1.138]). Analysis of the distribution of alleles and genotypes of the 5-HTTLPR polymorphism of the *5-HTT* gene showed the predominance of the short S allele (74.8–89.4%) and the SS genotype (61.7–81.5%) in both samples. Smokers had a significantly ($p<0.05$) higher frequency of the risk allele L (OR -2.830, 95% CI= [1.674 -4.783]) compared to non-smokers. When analyzing the 5-HTTLPR and rs25531 polymorphisms grouped into groups, it was found that the frequency of the L' allele was four times higher in the sample of smokers ($p<0.001$) than in the sample of non-smokers (OR -4.844, 95% CI = [2.503-9.372]). An analysis of the distribution of combinations of genotypes of both studied genes showed the predominance of people with a combination of GG genotypes of the *HTR2A* gene and S'S' of the *5-HTT* gene, which showed a protective effect on smoking (OR -0.550, 95% CI = [0.319-0.948]). A significant association with smoking was shown by a combination of heterozygous genotypes AG of the *HTR2A* gene and L'S' of the 5-HTT gene (OR -13.637, 95% CI= [1.752-106.144]). This study established a significant association of *5-HTT* gene polymorphisms with smoking in the Yakut population. In connection with the equivalent serotonin expression of the S and LG alleles, 5HTTLPR and rs25531 polymorphisms, it is more informative to carry out their generalized analysis.

Keywords: nicotine addiction, smoking, 5-HTT, HTR2A, rs6311, 5HTTLPR, rs25531, Yakut population

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